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## **Characterisation of hybrid larch somatic embryo maturation by biochemical analyses and by a novel, fast mid-infrared method.**

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The maturation of somatic embryos may present a slight variation in the timing of embryo development relative to each other. As the morphology of cotyledonary embryos is not a sufficient criterion to determine the level of maturation, analysing their biochemical composition could allow a precise definition of their physiological status. These analyses being very time-consuming and sample-consuming, we evaluated the relevance of using an alternative, rapid and economical method, which constitutes infrared spectrometry. We characterized hybrid larch somatic embryos biochemical composition throughout their development. In addition, these results were used to develop calibration models based on infrared spectrometry and chemometrics to provide new tools for the characterization of somatic embryos.

During the precotyledonary stage (0-3 weeks), the contents of simple carbohydrates, glucose and fructose, as well as the water content were high, consistent with the developmental stages. After 4 weeks of maturation, the accumulation of lipids, proteins and starch revealed a new orientation of somatic embryo metabolism. The presence of raffinose was only detected from 8 weeks of maturation. On the basis of these characteristics, calibration models were developed to predict on one hand the total water, protein, lipid and carbohydrate contents, and on the other hand for specific molecules of interest: glucose, fructose, inositol, raffinose, stachyose and starch. Since the developmental status is also a variable of major interest, we have also developed a model predicting the age of embryos, using the same spectra. This model takes into account the entire infrared spectrum and therefore all the components of the embryos. Only very slight variations appeared between the somatic embryos of 7 and 9 weeks of maturation. The prediction ability of this model, presented at least 72% of accuracy, the missed-class corresponded to attributions in neighboring classes, showing the similitude between embryos from closed age of maturation.

In conclusion, characterizing the biochemical composition of embryos throughout their development allows a better understanding of their maturation. But above all, these results offer a new, very rapid, sample-saving method for monitoring the maturation of somatic embryos by accessing a set of information on their composition contained in infrared spectra.